US PAT NO: **5,708,155** [IMAGE AVAILABLE] L1: 1 of 1

CLAIMS:

CLMS(1)

We claim:

1. A DNA construct encoding a chimeric protein, said DNA construct comprising a first nucleotide sequence encoding a leukotoxin polypeptide capable of activating helper T-cells directed to a selected antigen, operably linked to a second nucleotide sequence encoding said selected antigen.

CLMS(2)

2. The DNA construct of claim 1 wherein said second nucleotide sequence encodes somatostatin (SRIF), or an epitope thereof.

CLMS(3)

3. The DNA construct of claim 2 comprising the nucleotide sequence depicted in SEQ ID NO:9.

CLMS(4)

4. The DNA construct of claim 1 wherein said second nucleotide sequence encodes gonadotropin releasing hormone (GnRH) comprising the amino acid sequence Gln-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly, or an epitope thereof.

CLMS(5)

5. The DNA construct of claim 4 comprising the nucleotide sequence depicted in SEQ ID NO:11.

CLMS(6)

6. The DNA construct of claim 1 wherein said second nucleotide sequence encodes bovine rotavirus VP4, or an epitope thereof.

CLMS(7)

7. The DNA construct of claim 6 comprising the nucleotide sequence depicted in SEQ ID NO:13.

CLMS(8)

8. An expression cassette comprised of:
(a) the DNA construct of claim 1; and
(b) control sequences that direct the transcription of said construct whereby said construct can be transcribed and translated in a host cell.

CLMS(9)

9. An expression cassette comprised of:
(a) the DNA construct of claim 2; and
(b) control sequences that direct the transcription said construct whereby said construct can be transcribed and translated in a host

cell.

CLMS(10)

10. An expression cassette comprised of:
(a) the DNA construct of claim 4; and
(b) control sequences that direct the transcription said construct whereby said construct can be transcribed and translated in a host cell.

CLMS(11)

11. An expression cassette comprised of:
(a) the DNA construct of claim 6; and
(b) control sequences that direct the transcription said construct whereby said construct can be transcribed and translated in a host cell.

CLMS(12)

12. A host cell stably transformed with the expression cassette of claim 8.

CLMS(13)

13. A host cell stably transformed with the expression cassette of claim 9.

CLMS(14)

14. A host cell stably transformed with the plasmid of claim 10.

CLMS(15)

15. A host cell stably transformed with the plasmid of claim 11.

CLMS(16)

16. A method of producing a recombinant polypeptide comprising:
(a) providing a population of host cells according to claim 12; and
(b) growing said population of cells under conditions whereby the polypeptide encoded by said expression cassette is expressed.

CLMS(17)

17. A method of producing a recombinant polypeptide comprising:
(a) providing a population of host cells according to claim 13; and
(b) growing said population of cells under conditions whereby the polypeptide encoded by said expression cassette is expressed.

CLMS(18)

18. A method of producing a recombinant polypeptide comprising:
(a) providing a population of host cells according to claim 14; and
(b) growing said population of cells under conditions whereby the polypeptide encoded by said expression cassette is expressed.

CLMS(19)

19. A method of producing a recombinant polypeptide comprising:
(a) providing a population of host cells according to claim 15; and
(b) growing said population of cells under conditions whereby the polypeptide encoded by said expression cassette is expressed.

US PAT NO: 5,723,129 [IMAGE AVAILABLE] L3: 4 of 11

CLAIMS:

CLMS(1)

We claim:

1. A chimeric protein comprising a leukotoxin polypeptide fused to a multimer having more than one selected GnRH polypeptide, whereby said leukotoxin portion of said chimeric protein acts to increase the immunogenicity of said GnRH multimer.

CLMS(2)

2. The chimeric protein of claim 1 wherein said leukotoxin polypeptide lacks leukotoxic activity.

CLMS(3)

3. The chimeric protein of claim 2 wherein said leukotoxin is LKT 352.

CLMS(4)

4. The chimeric protein of claim 2 wherein said leukotoxin is LKT 111.

CLMS(5)

5. The chimeric protein of claim 1 wherein said GnRH multimer comprises a molecule according to the general formula GnRH-X-GnRH wherein X is selected from the group consisting of a peptide linkage, an amino acid spacer group, a leukotoxin polypeptide and [GnRH].sub.n where n is greater than or equal to 1, and further wherein GnRH comprises any GnRH polypeptide.

CLMS(6)

6. The chimeric protein of claim 5 wherein X comprises an amino acid spacer group including at least one helper T-cell epitope.

CLMS(7)

7. The chimeric protein of claim 1 wherein said chimeric protein comprises the amino acid sequence depicted in FIGS. 5A-5h, SEQ ID NOS:7-8.

CLMS(8)

8. The chimeric protein of claim 1 wherein said chimeric protein comprises the amino acid sequence depicted in FIGS. 7A-7E, SEQ ID NOS:9-10.

CLMS(9)

9. A vaccine composition comprising the chimeric protein of claim 1 and a pharmaceutically acceptable vehicle.

CLMS(10)

10. A vaccine composition comprising the chimeric protein of claim 2 and a pharmaceutically acceptable vehicle.

CLMS(11)

11. A vaccine composition comprising the chimeric protein of claim 5 and a pharmaceutically acceptable vehicle.

CLMS(12)

12. A vaccine composition comprising the chimeric protein of claim 7 and a pharmaceutically acceptable vehicle.

CLMS(13)

13. A vaccine composition comprising the chimeric protein of claim 8 and a pharmaceutically acceptable vehicle.

CLMS(14)

14. A method for presenting a selected GnRH multimer to a subject comprising administering to said subject an effective amount of a vaccine composition according to claim 9.

CLMS(15)

15. A method for presenting a selected GnRH multimer to a subject comprising administering to said subject an effective amount of a vaccine composition according to claim 10.

CLMS(16)

16. A method for presenting a selected GnRH multimer to a subject comprising administering to said subject an effective amount of a vaccine composition according to claim 11.

CLMS(17)

17. A method for presenting a selected GnRH multimer to a subject comprising administering to said subject an effective amount of a vaccine composition according to claim 12.

CLMS(18)

18. A method for presenting a selected GnRH multimer to a subject comprising administering to said subject an effective amount of a vaccine composition according to claim 13.

CLMS(19)

19. A chimeric protein comprising a leukotoxin polypeptide fused to a multimer having eight selected GnRH polypeptides, wherein the C-terminus of the leukotoxin polypeptide is fused to the N-terminus of the multimer.

CLMS(20)

20. The chimeric protein of claim 19, wherein the leukotoxin polypeptide comprises the 52 kD LKT 111 carrier polypeptide.

CLMS(21)

21. A chimeric protein comprising a leukotoxin polypeptide fused to a multimer having eight selected GnRH polypeptides, wherein the C-terminus of the multimer is fused to the N-terminus of the leukotoxin polypeptide.

CLMS(22)

US PAT NO: 5,837,268 [IMAGE AVAILABLE] L3: 1 of 11

CLAIMS:

CLMS(1)

We claim:

1. A chimeric protein comprising a leukotoxin polypeptide fused to first and second multimers, wherein the C-terminus of the first multimer is fused to the N-terminus of the leukotoxin polypeptide and the N-terminus of the second multimer is fused to the C-terminus of the leukotoxin polypeptide, and further wherein each of said multimers comprises more than one selected GnRH polypeptide.

CLMS(2)

2. The chimeric protein of claim 1 wherein the first and second GnRH multimers are different and comprise molecules according to the general formula [GnRH-X-GnRH].sub.n, wherein:
GnRH comprises a GnRH polypeptide;
X is selected from the group consisting of a peptide linkage, an amino acid spacer group and a leukotoxin polypeptide; and
n is an integer greater than or equal to 1.

CLMS(3)

3. The chimeric protein of claim 1 wherein the first and second GnRH multimers are the same and comprise molecules according to the general formula [GnRH-X-GnRH].sub.n, wherein:
GnRH comprises a GnRH polypeptide;
X is selected from the group consisting of a peptide linkage, an amino acid spacer group and a leukotoxin polypeptide; and
n is an integer greater than or equal to 1.

CLMS(4)

4. The chimeric protein of claim 3 wherein X is an amino acid spacer group having at least one helper T-cell epitope.

CLMS(5)

5. The chimeric protein of claim 3 wherein n is 4.

CLMS(6)

6. The chimeric protein of claim 1 wherein the leukotoxin polypeptide lacks cytotoxic activity.

CLMS(7)

7. The chimeric protein of claim 6 wherein the leukotoxin polypeptide is the polypeptide depicted at amino acid residues 11-923 of SEQ ID NO:6.

CLMS(8)

8. The chimeric protein of claim 6 wherein the leukotoxin polypeptide is the polypeptide depicted at amino acid residues 11-491 of SEQ ID NO:10.

CLMS(9)

9. The chimeric protein of claim 6 wherein the leukotoxin polypeptide is SEQ ID NO:17.

CLMS(10)

10. The chimeric protein of claim 3 wherein the first multimer further comprises the amino acid sequence (Met-Ala-Thr-Val-Ile-Asp-Arg-Ser SEQ ID NO:21) fused to the N-terminus thereof.

CLMS(11)

11. The chimeric protein of claim 1 comprising the amino acid sequence depicted in FIGS. 9-1 through 9-6 (SEQ ID NO:15 and SEQ ID NO:16).

CLMS(12)

12. A vaccine composition comprising the chimeric protein of claim 1 and a pharmaceutically acceptable vehicle.

CLMS(13)

13. A vaccine composition comprising the chimeric protein of claim 3 and a pharmaceutically acceptable vehicle.

CLMS(14)

14. A vaccine composition comprising the chimeric protein of claim 5 and a pharmaceutically acceptable vehicle.

CLMS(15)

15. A vaccine composition comprising the chimeric protein of claim 6 and a pharmaceutically acceptable vehicle.

CLMS(16)

16. A vaccine composition comprising the chimeric protein of claim 11 and a pharmaceutically acceptable vehicle.

CLMS(17)

17. A method for presenting selected GnRH multimers to a subject comprising administering to said subject an effective amount of the vaccine composition according to claim 12.

CLMS(18)

18. A method for presenting selected GnRH multimers to a subject comprising administering to said subject an effective amount of the vaccine composition according to claim 13.

CLMS(19)

19. A method for presenting selected GnRH multimers to a subject comprising administering to said subject an effective amount of the vaccine composition according to claim 14.

CLMS(20)

20. A method for presenting selected GnRH multimers to a subject comprising administering to said subject an effective amount of the vaccine composition according to claim 15.

CLMS(21)

21. A method for presenting selected GnRH multimers to a subject comprising administering to said subject an effective amount of the vaccine composition according to claim 16.

CLMS(22)

22. A method for reducing the incidence of mammary tumors in a mammalian subject comprising administering a therapeutically effective amount of the vaccine composition of claim 12 to said subject.

CLMS(23)

23. A method for reducing the incidence of mammary tumors in a mammalian subject comprising administering a therapeutically effective amount of the vaccine composition of claim 16 to said subject.

U

S PAT NO: **5,422,110** [IMAGE AVAILABLE] L2: 1 of 1

CLAIMS:

CLMS(1)

We claim:

1. An immunological carrier system comprising a chimeric protein, said chimeric protein consisting of a leukotoxin molecule which lacks leukotoxic activity, fused to somatostatin (SRIF), whereby said leukotoxin of said chimeric protein acts to increase the immunogenicity of said SRIF.

CLMS(2)

2. The carrier system of claim 1 wherein said chimetic protein consists of the amino acid sequence depicted in FIG. 6 (SEQ ID NO:8).

CLMS(3)

3. A vaccine composition comprising the chimetic protein of claim 1 and a pharmaceutically acceptable vehicle.

CLMS(4)

4. A method for presenting a selected antigen to a subject comprising administering to said subject an effective amount of a vaccine composition according to claim 3.

CLMS(5)

5. An immunological carrier system comprising a chimeric protein, said chimeric protein consisting of a leukotoxin molecule which lacks leukotoxic activity, fused to gonadotropin releasing hormone (GnRH), whereby said leukotoxin of said chimeric protein acts to increase the immunogenicity of said GnRH.

CLMS(6)

6. The carrier system of claim 5 wherein said chimeric protein consists of the amino acid sequence depicted in FIG. 8 (SEQ ID NO:9).

CLMS(7)

7. A vaccine composition comprising the chimeric protein of claim 5 and a pharmaceutically acceptable vehicle.

CLMS(8)

8. A method for presenting a selected antigen to a subject comprising administering to said subject an effective amount of a vaccine composition according to claim 7.

CLMS(9)

9. An immunological carrier system comprising a chimetic protein, said chimetic protein consisting of a leukotoxin molecule which lacks leukotoxic activity, fused to bovine rotavirus VP4, whereby said leukotoxin of said chimeric protein acts to increase the immunogenicity

of said VP4.

CLMS(10)

10. The carrier system of claim 9 wherein said chimetic protein consists of the amino acid sequence depicted in FIG. 10 (SEQ ID NO:10).

CLMS(11)

11. A vaccine composition comprising the chimetic protein of claim 9 and a pharmaceutically acceptable vehicle.

CLMS(12)

12. A method for presenting a selected antigen to a subject comprising administering to said subject an effective amount of a vaccine composition according to claim 11.

US PAT NO: **5,422,110** [IMAGE AVAILABLE] L1: 1 of 1
DATE ISSUED: Jun. 6, 1995
TITLE: Enhanced immunogenicity using leukotoxin chimeras
INVENTOR: Andrew A. Potter, Saskatchewan, Canada
Mark J. Redmond, Saskatchewan, Canada
Huw P. A. Hughes, Saskatchewan, Canada
ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign corp.)
APPL-NO: 07/960,932
DATE FILED: Oct. 14, 1992
REL-US-DATA: Continuation-in-part of Ser. No. 779,171, Oct. 16, 1991, abandoned.
INT-CL: [6] A61K 39/102; C12N 15/31
US-CL-ISSUED: 424/255.1, 184.1, 190.1, 192.1, 234.1; 530/350, 825; 435/69.3, 172.1, 172.3, 69.1; 536/23.4, 23.7
US-CL-CURRENT: 424/255.1, 184.1, 190.1, 192.1, 234.1; 435/69.1, 69.3; 530/350, 825; 536/23.4, 23.7
SEARCH-FLD: 530/350, 825; 424/88, 92, 184.1, 190.1, 192.1, 234.1, 255.1; 435/69.3, 172.1, 172.3, 69.7; 536/23.4, 23.7; 935/13, 47

REF-CITED:

U.S. PATENT DOCUMENTS

4,722,840	2/1988	Valenzuela et al.	
4,867,973	9/1989	Goers et al.	424/85.91
4,957,739	9/1990	Berget et al.	424/92
4,975,420	12/1990	Silversides et al.	514/15
5,028,423	7/1991	Prickett	
5,055,400	10/1991	Lo et al.	
5,238,823	8/1993	Potter et al.	435/69.52

FOREIGN PATENT DOCUMENTS

WO90/10458	9/1990	World Intellectual Property Organization
WO91/15237	10/1991	World Intellectual Property Organization
WO92/03558	3/1992	World Intellectual Property Organization

OTHER PUBLICATIONS

Phalipin, A. et al. Gene 55:255-263 (1987).
Buggemann, P. et al BioTechniques 10(2):202-209 (1991).
Forestier, C. et al. 1991. Infection and Immunity 59(11):4212-4220.
Sad, S. et al. 1991. Immunology 74:223-227.
Que et al. 1988. Infection & Immunity 56(10):2645-2649.
Bittle et al., Nature (1982) 298:30-33.
Muller et al., Proc. Natl. Acad. Sci. (1982) 79:569-573.
Schutze et al., J. Immunol. (1985) 135(4):2319-2322.
Lowell et al., Science (1988) 240:800-802.
Morein et al., Nature (1984) 308:457-460.
Neurath et al., Mol. Immunol. (1989) 26(1):53-62.
Redmond et al., Mol. Immunol. (1991) 28(3):269-278.
Kingsman and Kingsman, Vaccine (1988) 6:304-306.
Valenzuela et al., Bio/Technology (1985) 3:323-326.
Delpeyroux et al., Science (1986) 233:472-475.
Clarke et al., Vaccines 88 Ginsberg, H., et al., Eds., (1988) pp. 127-131.
Burke et al., Nature (1988) 332:81-82.
Haynes et al., Bio/Technology (1986) 4:637-641.
Gentry et al., Vet. Immunology and Immunopathology (1985) 9:239-250.
Strathdee and Lo, Infect. Immun. (1987) 55(12):3233-3236.

Lo et al., Infect. Immun. (1985) 50(3):667-671.
ART-UNIT: 183
PRIM-EXMR: Hazel F. Sidberry
ASST-EXMR: Michael S. Tuscan
LEGAL-REP: Reed & Robins

ABSTRACT:
New immunological carrier systems, DNA encoding the same, and the use of these systems, are disclosed. The carrier systems include chimeric proteins which comprise a leukotoxin polypeptide fused to a selected antigen. The leukotoxin functions to increase the immunogenicity of the antigen fused thereto.
12 Claims, 10 Drawing Figures

22. The chimeric protein of claim 21, wherein the leukotoxin polypeptide comprises the 52 kD LKT 111 carrier polypeptide.

CLMS(23)

23. A vaccine composition comprising the chimeric protein of claim 19 and a pharmaceutically acceptable vehicle.

CLMS(24)

24. A vaccine composition comprising the chimeric protein of claim 20 and a pharmaceutically acceptable vehicle.

CLMS(25)

25. A vaccine composition comprising the chimeric protein of claim 21 and a pharmaceutically acceptable vehicle.

CLMS(26)

26. A vaccine composition comprising the chimeric protein of claim 22 and a pharmaceutically acceptable vehicle.

US PAT NO: 5,476,657 [IMAGE AVAILABLE] L3: 8 of 11

CLAIMS:

CLMS(1)

I claim:

1. A vaccine composition comprising P. haemolytica leukotoxin 352 (LKT 352), as depicted in FIG. 5 and a pharmaceutically acceptable vehicle.

CLMS(2)

2. The vaccine composition of claim 1 wherein the composition further comprises a saline extract of P. haemolytica.

CLMS(3)

3. The vaccine composition of claim 1 further comprising an adjuvant.

CLMS(4)

4. The vaccine composition of claim 2 further comprising an adjuvant.

CLMS(5)

5. A vaccine composition comprising a pharmaceutically acceptable vehicle, an adjuvant, P. haemolytica leukotoxin 352 (LKT 352), as depicted in FIG. 5, and a saline extract of P. haemolytica.

CLMS(6)

6. Isolated P. haemolytica leukotoxin 352 (LKT 352), having the amino acid sequence depicted in FIG. 5.

CLMS(7)

7. A method for preventing or ameliorating respiratory disease in a ruminant subject, said method comprising administering an amount of a vaccine composition according to claim 1 effective to produce an immunological response, to said ruminant subject.

8. A method for preventing or ameliorating respiratory disease in a ruminant subject, said method comprising administering an amount of a vaccine composition according to claim 4 effective to produce an immunological response, to said ruminant subject.

(b) control sequences that direct the transcription said construct whereby said construct can be transcribed and translated in a host cell.

CLMS(12)

12. A host cell stably transformed with the expression cassette of claim 8.

CLMS(13)

13. A host cell stably transformed with the expression cassette of claim 9.

CLMS(14)

14. A host cell stably transformed with the plasmid of claim 10.

CLMS(15)

15. A host cell stably transformed with the plasmid of claim 11.

CLMS(16)

16. A method of producing a recombinant polypeptide comprising:
(a) providing a population of host cells according to claim 12; and
(b) growing said population of cells under conditions whereby the polypeptide encoded by said expression cassette is expressed.

CLMS(17)

17. A method of producing a recombinant polypeptide comprising:
(a) providing a population of host cells according to claim 13; and
(b) growing said population of cells under conditions whereby the polypeptide encoded by said expression cassette is expressed.

CLMS(18)

18. A method of producing a recombinant polypeptide comprising:
(a) providing a population of host cells according to claim 14; and
(b) growing said population of cells under conditions whereby the polypeptide encoded by said expression cassette is expressed.

CLMS(19)

19. A method of producing a recombinant polypeptide comprising:
(a) providing a population of host cells according to claim 15; and
(b) growing said population of cells under conditions whereby the polypeptide encoded by said expression cassette is expressed.

US PAT NO: 5,476,657 [IMAGE AVAILABLE] L4: 21 of 35

CLAIMS:

CLMS(1)

I claim:

1. A vaccine composition comprising P. haemolytica **leukotoxin** 352 (LKT 352), as depicted in FIG. 5 and a pharmaceutically acceptable vehicle.

CLMS(2)

2. The vaccine composition of claim 1 wherein the composition further comprises a saline extract of P. haemolytica.

CLMS(3)

3. The vaccine composition of claim 1 further comprising an adjuvant.

CLMS(4)

4. The vaccine composition of claim 2 further comprising an adjuvant.

CLMS(5)

5. A vaccine composition comprising a pharmaceutically acceptable vehicle, an adjuvant, P. haemolytica **leukotoxin** 352 (LKT 352), as depicted in FIG. 5, and a saline extract of P. haemolytica.

CLMS(6)

6. Isolated P. haemolytica **leukotoxin** 352 (LKT 352), having the amino acid sequence depicted in FIG. 5.

CLMS(7)

7. A method for preventing or ameliorating respiratory disease in a ruminant subject, said method comprising administering an amount of a vaccine composition according to claim 1 effective to produce an immunological response, to said ruminant subject.

CLMS(8)

8. A method for preventing or ameliorating respiratory disease in a ruminant subject, said method comprising administering an amount of a vaccine composition according to claim 4 effective to produce an immunological response, to said ruminant subject.

US PAT NO: 5,378,615 [IMAGE AVAILABLE] L4: 26 of 35

CLAIMS:

CLMS(1)

The embodiments of the invention in which an exclusive property or provilege is claimed are defined as follows:

1. A process for producing a non-toxic inactive cytotoxin specific for ruminant leukocytes comprising the steps of:
(A) culturing an inoculum of Pasteurella haemolytica having an optical density of about 0.18 measured at a wavelength of 525 nm, in a serum-free medium for a period in the range of 1.5 to 3 hrs, so as to produce said cytotoxin;
(B) periodically measuring the optical density of said serum-free medium;
(C) upon detecting a value for the optical density of about 0.37, measured at a wavelength of 525 nm, which indicates the phase of logarithmic growth of the cells when an optimum concentration of cytotoxin is produced in said serum-free medium, separating supernatant liquid containing said cytotoxin from the resulting culture;
(D) separating solids, including any of said cells, from the resulting supernatant liquid so as to obtain a Pasteurella haemolytica serum-free, cell-free solution of said cytotoxin which is essentially endotoxin-free.

CLMS(2)

2. The process of claim 1, additionally comprising step (E):
adding serum to the resulting solution of step (D) so as to stabilize said cytotoxin for the purpose of analysis of toxic activity.

S708/55

1. A DNA construct encoding a chimeric protein, said DNA construct comprising a first nucleotide sequence encoding a **leukotoxin** polypeptide capable of activating helper T-cells directed to a selected antigen, operably linked to a second nucleotide sequence encoding said selected antigen.

CLMS(2)

2. The DNA construct of claim 1 wherein said second nucleotide sequence encodes somatostatin (SRIF), or an epitope thereof.

CLMS(3)

3. The DNA construct of claim 2 comprising the nucleotide sequence depicted in SEQ ID NO:9.

CLMS(4)

4. The DNA construct of claim 1 wherein said second nucleotide sequence encodes gonadotropin releasing hormone (GnRH) comprising the amino acid sequence Gln-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly, or an epitope thereof.

CLMS(5)

5. The DNA construct of claim 4 comprising the nucleotide sequence depicted in SEQ ID NO:11.

CLMS(6)

6. The DNA construct of claim 1 wherein said second nucleotide sequence encodes bovine rotavirus VP4, or an epitope thereof.

CLMS(7)

7. The DNA construct of claim 6 comprising the nucleotide sequence depicted in SEQ ID NO:13.

CLMS(8)

8. An expression cassette comprised of:
(a) the DNA construct of claim 1; and
(b) control sequences that direct the transcription of said construct whereby said construct can be transcribed and translated in a host cell.

CLMS(9)

9. An expression cassette comprised of:
(a) the DNA construct of claim 2; and
(b) control sequences that direct the transcription said construct whereby said construct can be transcribed and translated in a host cell.

CLMS(10)

10. An expression cassette comprised of:
(a) the DNA construct of claim 4; and
(b) control sequences that direct the transcription said construct whereby said construct can be transcribed and translated in a host cell.

CLMS(11)

11. An expression cassette comprised of:
(a) the DNA construct of claim 6; and

L4 35 LEUKOTOXIN

=> d bib 1-35

L4: 1 of 35

US PAT NO: 5,837,268 [IMAGE AVAILABLE]
DATE ISSUED: Nov. 17, 1998
TITLE: GnRH-**leukotoxin** chimeras
INVENTOR: Andrew A. Potter, Saskatoon, Canada
John G. Manns, Saskatoon, Canada
ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign corp.)
APPL-NO: 08/694,865
DATE FILED: Aug. 9, 1996
ART-UNIT: 165
PRIM-EXMR: Nita Minnifield
LEGAL-REP: Robins & Associates

L4: 2 of 35

US PAT NO: 5,824,525 [IMAGE AVAILABLE]
DATE ISSUED: Oct. 20, 1998
TITLE: Construction of Pasteurella haemolytica vaccines
INVENTOR: Robert E. Briggs, Boone, IA
Fred M. Tatum, Ames, IA
ASSIGNEE: Biotechnology Research and Development Corporation, Peoria, IL (U.S. corp.)
The United States of America as represented by the Department of Agriculture, Washington, DC (U.S. govt.)
APPL-NO: 08/643,301
DATE FILED: May 8, 1996
ART-UNIT: 162
PRIM-EXMR: Eric Grimes
LEGAL-REP: Banner & Witcoff, Ltd.

L4: 3 of 35

US PAT NO: 5,804,190 [IMAGE AVAILABLE]
DATE ISSUED: Sep. 8, 1998
TITLE: Recombinant vaccine for porcine pleuropneumonia
INVENTOR: Douglas K. Struck, College Station, TX
Ryland F. Young, College Station, TX
Yung-Fu Chang, Ithaca, NY
ASSIGNEE: The Texas A&M University System, College Station, TX (U.S. corp.)
APPL-NO: 08/850,379
DATE FILED: May 2, 1997
ART-UNIT: 163
PRIM-EXMR: Mary E. Mosher
LEGAL-REP: Arnold, White & Durkee

L4: 4 of 35

US PAT NO: 5,801,018 [IMAGE AVAILABLE]
DATE ISSUED: Sep. 1, 1998
TITLE: Vaccines for Actinobacillus pleuropneumoniae
INVENTOR: Andrew A. Potter, Saskatoon, Canada
Gerald F. Gerlach, Saskatoon, Canada
Philip J. Willson, Saskatoon, Canada
Amalia Rossi-Campos, Saskatoon, Canada
ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign

corp.)
APPL-NO: 08/321,978
DATE FILED: Oct. 12, 1994
ART-UNIT: 163
PRIM-EXMR: Mary E. Mosher
LEGAL-REP: Robins & Associates

L4: 5 of 35

US PAT NO: 5,789,184 [IMAGE AVAILABLE]
DATE ISSUED: Aug. 4, 1998
TITLE: Yeast cells engineered to produce pheromone system protein surrogates, and uses therefor
INVENTOR: Dana M. Fowlkes, Chapel Hill, NC
Jim Broach, Princeton, NJ
John Manfredi, Ossining, NY
Christine Klein, Ossining, NY
Andrew J. Murphy, Montclair, NJ
Jeremy Paul, South Nyack, NY
Joshua Trueheart, South Nyack, NY
ASSIGNEE: Cadus Pharmaceutical Corporation, Tarrytown, NY (U.S. corp.)
APPL-NO: 08/464,531
DATE FILED: Jun. 5, 1995
ART-UNIT: 166
PRIM-EXMR: James Ketter
ASST-EXMR: Irem Yucel
LEGAL-REP: Giulio A. DeConti, Jr., Catherine J. Kara

L4: 6 of 35

US PAT NO: 5,783,195 [IMAGE AVAILABLE]
DATE ISSUED: Jul. 21, 1998
TITLE: Recombinant infectious bovine rhinotracheitis virus S-IBR-052 and uses thereof
INVENTOR: Mark D. Cochran, Carlsbad, CA
Richard D. Macdonald, San Diego, CA
ASSIGNEE: Syntro Corporation, Lenexa, KS (U.S. corp.)
APPL-NO: 08/191,866
DATE FILED: Feb. 4, 1994
ART-UNIT: 185
PRIM-EXMR: Nancy Degen
ASST-EXMR: Terry A. McKelvey
LEGAL-REP: John P.Cooper & Dunham LLP White

L4: 7 of 35

US PAT NO: 5,733,780 [IMAGE AVAILABLE]
DATE ISSUED: Mar. 31, 1998
TITLE: Construction of Pasteurella haemolytica vaccines
INVENTOR: Robert E. Briggs, Boone, IA
Fred M. Tatum, Ames, IA
ASSIGNEE: The United States of America as represented by the Department of Agriculture, Washington, DC (U.S. govt.)
Biotechnology and Research and Development Corporation, Poria, IL (U.S. corp.)
APPL-NO: 08/643,298
DATE FILED: May 8, 1996
ART-UNIT: 185
PRIM-EXMR: Nancy T. Vogel
LEGAL-REP: Banner & Witcoff, Ltd.

L4: 8 of 35

US PAT NO: 5,726,016 [IMAGE AVAILABLE]
DATE ISSUED: Mar. 10, 1998
TITLE: Compositions and methods for diagnosis of diseases associated with actinobacillus actinomycetemcomitans infection
INVENTOR: Donald R. DeMuth, Drexel Hill, PA

ASSIGNEE: Edward T. Lally, West Chester, PA
The Trustees of the University of Pennsylvania, Philadelphia, PA (U.S. corp.)
APPL-NO: 08/374,843
DATE FILED: Jan. 18, 1995
ART-UNIT: 187
PRIM-EXMR: Carla J. Myers
LEGAL-REP: Panitch Schwarze Jacobs & Nadel, P.C.

L4: 9 of 35

US PAT NO: 5,723,129 [IMAGE AVAILABLE]
DATE ISSUED: Mar. 3, 1998
TITLE: GnRH-**leukotoxin** chimeras
INVENTOR: Andrew A. Potter, Saskatoon, Canada
John G. Manns, Saskatoon, Canada
ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign corp.)
APPL-NO: 08/387,156
DATE FILED: Feb. 10, 1995
ART-UNIT: 187
PRIM-EXMR: N. M. Minnifield
LEGAL-REP: Robins & Associates

L4: 10 of 35

US PAT NO: 5,708,155 [IMAGE AVAILABLE]
DATE ISSUED: Jan. 13, 1998
TITLE: Enhanced immunogenicity using **leukotoxin** chimeras
INVENTOR: Andrew A. Potter, Saskatoon, Canada
Mark J. Redmond, Saskatoon, Canada
Huw P. A. Hughes, Saskatoon, Canada
ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign corp.)
APPL-NO: 08/455,970
DATE FILED: May 31, 1995
ART-UNIT: 182
PRIM-EXMR: James C. Housel
ASST-EXMR: Jennifer Shaver
LEGAL-REP: Robins & Associates

L4: 11 of 35

US PAT NO: 5,693,777 [IMAGE AVAILABLE]
DATE ISSUED: Dec. 2, 1997
TITLE: DNA encoding pasteurella haemolytica PhaI restriction endonuclease and methyltransterase
INVENTOR: Robert E. Briggs, Boone, IA
Fred M. Tatum, Ames, IA
ASSIGNEE: The United States of America as represented by the Secretary of Agriculture, Washington, DC (U.S. govt.)
Biotechnology Research and Development Corporation, Peoria, IL (U.S. corp.)
APPL-NO: 08/643,297
DATE FILED: May 8, 1996
ART-UNIT: 189
PRIM-EXMR: John L. LeGuyader
LEGAL-REP: Banner & Witcoff, Ltd.

L4: 12 of 35

US PAT NO: 5,683,900 [IMAGE AVAILABLE]
DATE ISSUED: Nov. 4, 1997
TITLE: Pasteurella haemolytica PhaI restriction endonuclcape and methyltranstesase
INVENTOR: Robert E. Briggs, Boone, IA
Fred M. Tatum, Ames, IA
ASSIGNEE: The United States of America as represented by the Department of Agriculture, Washington, DC (U.S. govt.)
Biotechnology Research and Development Corporation,

Peoria, IL (U.S. corp.)
APPL-NO: 08/643,300
DATE FILED: May 8, 1996
ART-UNIT: 189
PRIM-EXMR: John L. LeGuyader
LEGAL-REP: Banner & Witcoff, Ltd.

US PAT NO: 5,641,653 [IMAGE AVAILABLE] L4: 13 of 35
DATE ISSUED: Jun. 24, 1997
TITLE: DNA encoding Actinobacillus pleuropneumoniae hemolysin
INVENTOR: Douglas K. Struck, College Station, TX
Ryland F. Young, College Station, TX
Yung-Fu Chang, Ithaca, NY
ASSIGNEE: The Texas A&M University System, College Station, TX (U.S. corp.)
APPL-NO: 07/429,273
DATE FILED: Oct. 31, 1989
ART-UNIT: 185
PRIM-EXMR: Mary E. Mosher
LEGAL-REP: Arnold, White & Durkee

US PAT NO: 5,594,107 [IMAGE AVAILABLE] L4: 14 of 35
DATE ISSUED: Jan. 14, 1997
TITLE: Chimeric protein comprising an RTX-family cytotoxin and interferon-2 or interferon
INVENTOR: Andrew Potter, Saskatoon, Canada
Manuel Campos, Lincoln, NE
Huw P. A. Hughes, Saskatoon, Canada
ASSIGNEE: University of Saskatchewan, Saskatchewan, Canada (foreign corp.)
Ciba-Geigy Canada Ltd., Mississauga, Canada (foreign corp.)
APPL-NO: 08/170,126
DATE FILED: Dec. 20, 1993
ART-UNIT: 182
PRIM-EXMR: Stephen G. Walsh
ASST-EXMR: Lorraine M. Spector
LEGAL-REP: Reed & Robins

US PAT NO: 5,587,305 [IMAGE AVAILABLE] L4: 15 of 35
DATE ISSUED: Dec. 24, 1996
TITLE: Pasteurella haemolytica transformants
INVENTOR: Robert E. Briggs, Boone, IA
Fred M. Tatum, Ames, IA
ASSIGNEE: The United States of America as represented by the Department of Agriculture, Washington, DC (U.S. govt.)
Biotechnology Research and Development Corporation, Peoria, IL (U.S. corp.)
APPL-NO: 08/162,392
DATE FILED: Dec. 6, 1993
ART-UNIT: 185
PRIM-EXMR: John L. LeGuyader
LEGAL-REP: Banner & Allegretti, Ltd.

US PAT NO: 5,559,008 [IMAGE AVAILABLE] L4: 16 of 35
DATE ISSUED: Sep. 24, 1996
TITLE: **Leukotoxin** gene from Pasteurella suis
INVENTOR: Yung-Fu Chang, Ithaca, NY
ASSIGNEE: Cornell Research Foundation, Inc., Ithaca, NY (U.S. corp.)
APPL-NO: 08/215,805
DATE FILED: Mar. 22, 1994
ART-UNIT: 183

PRIM-EXMR: Mary E. Mosher
LEGAL-REP: Nixon, Hargrave, Devans & Doyle

US PAT NO: 5,543,312 [IMAGE AVAILABLE] L4: 17 of 35
DATE ISSUED: Aug. 6, 1996
TITLE: Pastuerella haemolytica glycoprotease gene and the purified enzyme
INVENTOR: Alan Mellors, Guelph, Canada
Reggie Y. C. Lo, Guelph, Canada
Khalid M. Abdullah, Kitchener, Canada
ASSIGNEE: University of Guelph, Ontario, Canada (foreign corp.)
APPL-NO: 08/087,797
DATE FILED: Aug. 12, 1993
ART-UNIT: 184
PRIM-EXMR: Robert A. Wax
ASST-EXMR: Eric Grimes
LEGAL-REP: Bell, Seltzer, Park & Gibson, P.A.

US PAT NO: 5,534,256 [IMAGE AVAILABLE] L4: 18 of 35
DATE ISSUED: Jul. 9, 1996
TITLE: Haemophilus somnus outer membrane protein extract enriched with iron-regulated proteins
INVENTOR: Andrew A. Potter, Saskatoon, Canada
Richard J. Harland, Saskatoon, Canada
ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign corp.)
APPL-NO: 07/908,253
DATE FILED: Jul. 2, 1992
ART-UNIT: 183
PRIM-EXMR: Hazel F. Sidberry
LEGAL-REP: Reed & Robins

US PAT NO: 5,521,072 [IMAGE AVAILABLE] L4: 19 of 35
DATE ISSUED: May 28, 1996
TITLE: Actinobacillus pleuropneumoniae transferrin binding proteins and uses thereof
INVENTOR: Andrew A. Potter, Saskatoon, Canada
Gerald F. Gerlach, Saskatoon, Canada
Philip J. Willson, Saskatoon, Canada
Amalia Rossi-Campos, Saskatoon, Canada
ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign corp.)
APPL-NO: 08/217,438
DATE FILED: Mar. 22, 1994
ART-UNIT: 183
PRIM-EXMR: Mary E. Mosher
LEGAL-REP: Reed & Robins

US PAT NO: 5,492,694 [IMAGE AVAILABLE] L4: 20 of 35
DATE ISSUED: Feb. 20, 1996
TITLE: Fusobacterium leukotoxoid vaccine
INVENTOR: Tiruvoor G. Nagaraja, Manhattan, KS
Muckatira M. Chengappa, Manhattan, KS
ASSIGNEE: Kansas State University Research Foundation, Manhattan, KS (U.S. corp.)
APPL-NO: 08/333,767
DATE FILED: Nov. 3, 1994
ART-UNIT: 188
PRIM-EXMR: Herbert J. Lilling
LEGAL-REP: Hovey, Williams, Timmons & Collins

US PAT NO: 5,476,657 [IMAGE AVAILABLE] L4: 21 of 35
DATE ISSUED: Dec. 19, 1995
TITLE: Pasteurella haemolytica **leukotoxin** compositions and uses thereof
INVENTOR: Andrew A. Potter, Saskatoon, Canada
ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign corp.)
APPL-NO: 08/015,537
DATE FILED: Feb. 9, 1993
ART-UNIT: 183
PRIM-EXMR: Hazel F. Sidberry
LEGAL-REP: Reed & Robins

US PAT NO: 5,462,735 [IMAGE AVAILABLE] L4: 22 of 35
DATE ISSUED: Oct. 31, 1995
TITLE: Pasteurella haemolytica subunit vaccine containing capsular polysaccharide and muramyl dipeptide
INVENTOR: Kim A. Brogden, Boone, IA
Louis Chedid, Tampa, FL
ASSIGNEE: The United States of America as represented by the Secretary of Agriculture, Washington, DC (U.S. govt.)
APPL-NO: 08/075,064
DATE FILED: Jun. 10, 1993
ART-UNIT: 183
PRIM-EXMR: Kay K. A. Kim
LEGAL-REP: M. Howard Silverstein, Curtis P. Ribando, John D. Fado

US PAT NO: 5,455,034 [IMAGE AVAILABLE] L4: 23 of 35
DATE ISSUED: Oct. 3, 1995
TITLE: Fusobacterium necrophorum leukotoxoid vaccine
INVENTOR: Tiruvoor G. Nagaraja, Manhattan, KS
Muckatira M. Chengappa, Manhattan, KS
ASSIGNEE: Kansas State University Research Foundation, Manhattan, KS (U.S. corp.)
APPL-NO: 08/078,066
DATE FILED: Jun. 18, 1993
ART-UNIT: 188
PRIM-EXMR: Herbert J. Lilling
LEGAL-REP: Hovey, Williams, Timmons & Collins

US PAT NO: 5,422,110 [IMAGE AVAILABLE] L4: 24 of 35
DATE ISSUED: Jun. 6, 1995
TITLE: Enhanced immunogenicity using **leukotoxin** chimeras
INVENTOR: Andrew A. Potter, Saskatchewan, Canada
Mark J. Redmond, Saskatchewan, Canada
Huw P. A. Hughes, Saskatchewan, Canada
ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign corp.)
APPL-NO: 07/960,932
DATE FILED: Oct. 14, 1992
ART-UNIT: 183
PRIM-EXMR: Hazel F. Sidberry
ASST-EXMR: Michael S. Tuscan
LEGAL-REP: Reed & Robins

US PAT NO: 5,417,971 [IMAGE AVAILABLE] L4: 25 of 35
DATE ISSUED: May 23, 1995
TITLE: Vaccines for Actinobacillus pleuropneumoniae
INVENTOR: Andrew A. Potter, Saskatoon, Canada
Gerald F. Gerlach, Saskatoon, Canada
Philip J. Willson, Saskatoon, Canada
Amalia Rossi-Campos, Saskatoon, Canada

ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign corp.)
APPL-NO: 07/961,522
DATE FILED: Oct. 15, 1992
ART-UNIT: 183
PRIM-EXMR: Christine M. Nucker
ASST-EXMR: Chris Dubrule
LEGAL-REP: Roberta L. Robins

US PAT NO: 5,378,615 [IMAGE AVAILABLE] L4: 26 of 35
DATE ISSUED: Jan. 3, 1995
TITLE: Process for the production of vaccine for prevention of Pasteurella haemolytica pneumonia in bovine
INVENTOR: Patricia E. Shewen, Guelph, Canada
Bruce N. Wilkie, Puslinch, Canada
ASSIGNEE: The University of Guelph, Canada (foreign corp.)
APPL-NO: 07/958,796
DATE FILED: Oct. 9, 1992
ART-UNIT: 188
PRIM-EXMR: Irene Marx
LEGAL-REP: Sughrue, Mion, Zinn, Macpeak & Seas

US PAT NO: 5,352,448 [IMAGE AVAILABLE] L4: 27 of 35
DATE ISSUED: Oct. 4, 1994
TITLE: Oral administration of antigens
INVENTOR: Terry L. Bowersock, Lafayette, IN
Waleed S. W. Shalaby, Mt. Pleasant, SC
William E. Blevins, Offerbem, IN
Michel Levy, West Lafayette, IN
Kinam Park, West Lafayette, IN
ASSIGNEE: Purdue Research Foundatioin, West Lafayette, IN (U.S. corp.)
APPL-NO: 07/916,533
DATE FILED: Jul. 20, 1992
ART-UNIT: 183
PRIM-EXMR: Christine M. Nucker
ASST-EXMR: Chris Dubrule
LEGAL-REP: Barnes & Thornburg

US PAT NO: 5,336,491 [IMAGE AVAILABLE] L4: 28 of 35
DATE ISSUED: Aug. 9, 1994
TITLE: Methods and compositions for the treatment and diagnosis of shipping fever
INVENTOR: Peter Berget, Pittsburgh, PA
Michael Engler, Houston, TX
Sarah Highlander, Houston, TX
George Weinstock, Houston, TX
ASSIGNEE: Board of Regents, The University of Texas System, Austin, TX (U.S. corp.)
APPL-NO: 07/899,100
DATE FILED: Jun. 15, 1992
ART-UNIT: 183
PRIM-EXMR: Christine M. Nucker
ASST-EXMR: H. F. Sidberry
LEGAL-REP: Arnold, White & Durkee

US PAT NO: 5,273,889 [IMAGE AVAILABLE] L4: 29 of 35
DATE ISSUED: Dec. 28, 1993
TITLE: Gamma-iterferon-**leukotoxin** gene fusions and uses thereof
INVENTOR: Andrew Potter, Saskatoon, Canada
Manuel Campos, Saskatoon, Canada

Huw P. A. Hughes, Saskatoon, Canada
ASSIGNEE: University of Saskatchewan, Saskatoon, Canada (foreign corp.)
Ciba-Geigy Canada, Ltd., Saskatoon, Canada (foreign corp.)
APPL-NO: 07/777,715
DATE FILED: Oct. 16, 1991
ART-UNIT: 182
PRIM-EXMR: Garnette D. Draper
LEGAL-REP: Reed & Robins

US PAT NO: 5,238,823 [IMAGE AVAILABLE] L4: 30 of 35
DATE ISSUED: Aug. 24, 1993
TITLE: Interleukin-2-**leukotoxin** gene fusions and uses thereof
INVENTOR: Andrew Potter, Saskatoon, Canada
Manuel Campos, Saskatoon, Canada
Huw P. A. Hughes, Saskatoon, Canada
ASSIGNEE: Veterinary Infectious Disease Organization, Saskatoon, Canada (foreign corp.)
Ciba-Geigy Canada Ltd, Mississauga, Canada (foreign corp.)
APPL-NO: 07/571,301
DATE FILED: Aug. 22, 1990
ART-UNIT: 182
PRIM-EXMR: Garnette D. Draper
LEGAL-REP: Reed & Robins

US PAT NO: 5,165,924 [IMAGE AVAILABLE] L4: 31 of 35
DATE ISSUED: Nov. 24, 1992
TITLE: Serum-free, cell-free vaccine effective against pneumonic pasteurellosis in cattle
INVENTOR: Patricia E. Shewen, Ontario, Canada
Bruce N. Wilkie, Ontario, Canada
ASSIGNEE: University of Guelph, Canada (foreign corp.)
APPL-NO: 07/462,929
DATE FILED: Jan. 12, 1990
ART-UNIT: 188
PRIM-EXMR: Irene Marx
LEGAL-REP: Sughrue, Mion, Zinn, Macpeak & Seas

US PAT NO: 5,055,400 [IMAGE AVAILABLE] L4: 32 of 35
DATE ISSUED: Oct. 8, 1991
TITLE: **Leukotoxin** gene of pasteurella haemolytica
INVENTOR: Reggie Y. C. Lo, Guelph, Canada
Patricia E. Shewen, Guelph, Canada
Craig A. Strathdee, Mississauga, Canada
ASSIGNEE: University of Guelph, Ontario, Canada (foreign corp.)
APPL-NO: 06/935,493
DATE FILED: Nov. 26, 1986
ART-UNIT: 184
PRIM-EXMR: Elizabeth C. Weimar
ASST-EXMR: Christopher Low
LEGAL-REP: Sughrue, Mion, Zinn Macpeak & Seas

US PAT NO: 5,028,423 [IMAGE AVAILABLE] L4: 33 of 35
DATE ISSUED: Jul. 2, 1991
TITLE: Immunogenic conjugates comprising **leukotoxin** peptide fragments
INVENTOR: Kathryn S. Prickett, Seattle, WA
ASSIGNEE: Immunex Corporation, Seattle, WA (U.S. corp.)
APPL-NO: 07/212,804
DATE FILED: Jun. 29, 1988
ART-UNIT: 189
PRIM-EXMR: John Doll

ASST-EXMR: Christina Chan

US PAT NO: 4,957,739 [IMAGE AVAILABLE] L4: 34 of 35
DATE ISSUED: Sep. 18, 1990
TITLE: Pharmaceutical compositions of a 105 kD P. Haemolytica derived antigen useful for treatment of Shipping Fever
INVENTOR: Peter Berget, Pittsburg, PA
Michael Engler, Houston, TX
Sarah Highlander, Houston, TX
George Weinstock, Houston, TX
ASSIGNEE: Board of Regents, The University of Texas System, Austin, TX (U.S. corp.)
APPL-NO: 07/085,430
DATE FILED: Aug. 13, 1987
ART-UNIT: 186
PRIM-EXMR: Garnette Draper
ASST-EXMR: Jeff Kushan
LEGAL-REP: Arnold, White & Durkee

US PAT NO: 4,857,516 [IMAGE AVAILABLE] L4: 35 of 35
DATE ISSUED: Aug. 15, 1989
TITLE: Coumaran derivatives and their pharmaceutical use
INVENTOR: Shinji Terao, Osaka, Japan
Yoshitaka Maki, Highland Park, IL
ASSIGNEE: Takeda Chemical Industries, Ltd., Osaka, Japan (foreign corp.)
APPL-NO: 07/136,273
DATE FILED: Dec. 22, 1987
ART-UNIT: 121
PRIM-EXMR: Mary C. Lee
ASST-EXMR: Bernard I. Dentz
LEGAL-REP: Wegner & Bretschneider

US PAT NO: 5,837,268 [IMAGE AVAILABLE] L4: 1 of 35

CLAIMS:

CLMS(1)

We claim:

1. A chimeric protein comprising a **leukotoxin** polypeptide fused to first and second multimers, wherein the C-terminus of the first multimer is fused to the N-terminus of the **leukotoxin** polypeptide and the N-terminus of the second multimer is fused to the C-terminus of the **leukotoxin** polypeptide, and further wherein each of said multimers comprises more than one selected GnRH polypeptide.

CLMS(2)

2. The chimeric protein of claim 1 wherein the first and second GnRH multimers are different and comprise molecules according to the general formula [GnRH-X-GnRH].sub.n, wherein:
GnRH comprises a GnRH polypeptide;
X is selected from the group consisting of a peptide linkage, an amino acid spacer group and a **leukotoxin** polypeptide; and
n is an integer greater than or equal to 1.

CLMS(3)

3. The chimeric protein of claim 1 wherein the first and second GnRH multimers are the same and comprise molecules according to the general formula [GnRH-X-GnRH].sub.n, wherein:
GnRH comprises a GnRH polypeptide;
X is selected from the group consisting of a peptide linkage, an amino acid spacer group and a **leukotoxin** polypeptide; and
n is an integer greater than or equal to 1.

CLMS(4)

4. The chimeric protein of claim 3 wherein X is an amino acid spacer group having at least one helper T-cell epitope.

CLMS(5)

5. The chimeric protein of claim 3 wherein n is 4.

CLMS(6)

6. The chimeric protein of claim 1 wherein the **leukotoxin** polypeptide lacks cytotoxic activity.

CLMS(7)

7. The chimeric protein of claim 6 wherein the **leukotoxin** polypeptide is the polypeptide depicted at amino acid residues 11-923 of SEQ ID NO:6.

CLMS(8)

8. The chimeric protein of claim 6 wherein the **leukotoxin** polypeptide is the polypeptide depicted at amino acid residues 11-491 of

SEQ ID NO:10.

CLMS(9)

9. The chimeric protein of claim 6 wherein the **leukotoxin** polypeptide is SEQ ID NO:17.

CLMS(10)

10. The chimeric protein of claim 3 wherein the first multimer further comprises the amino acid sequence (Met-Ala-Thr-Val-Ile-Asp-Arg-Ser SEQ ID NO:21) fused to the N-terminus thereof.

CLMS(11)

11. The chimeric protein of claim 1 comprising the amino acid sequence depicted in FIGS. 9-1 through 9-6 (SEQ ID NO:15 and SEQ ID NO:16).

CLMS(12)

12. A vaccine composition comprising the chimeric protein of claim 1 and a pharmaceutically acceptable vehicle.

CLMS(13)

13. A vaccine composition comprising the chimeric protein of claim 3 and a pharmaceutically acceptable vehicle.

CLMS(14)

14. A vaccine composition comprising the chimeric protein of claim 5 and a pharmaceutically acceptable vehicle.

CLMS(15)

15. A vaccine composition comprising the chimeric protein of claim 6 and a pharmaceutically acceptable vehicle.

CLMS(16)

16. A vaccine composition comprising the chimeric protein of claim 11 and a pharmaceutically acceptable vehicle.

CLMS(17)

17. A method for presenting selected GnRH multimers to a subject comprising administering to said subject an effective amount of the vaccine composition according to claim 12.

CLMS(18)

18. A method for presenting selected GnRH multimers to a subject comprising administering to said subject an effective amount of the vaccine composition according to claim 13.

CLMS(19)

19. A method for presenting selected GnRH multimers to a subject comprising administering to said subject an effective amount of the vaccine composition according to claim 14.

CLMS(20)

20. A method for presenting selected GnRH multimers to a subject comprising administering to said subject an effective amount of the vaccine composition according to claim 15.

CLMS(21)

21. A method for presenting selected GnRH multimers to a subject comprising administering to said subject an effective amount of the vaccine composition according to claim 16.

CLMS(22)

22. A method for reducing the incidence of mammary tumors in a mammalian subject comprising administering a therapeutically effective amount of the vaccine composition of claim 12 to said subject.

CLMS(23)

23. A method for reducing the incidence of mammary tumors in a mammalian subject comprising administering a therapeutically effective amount of the vaccine composition of claim 16 to said subject.

US PAT NO: 5,824,525 [IMAGE AVAILABLE] L4: 2 of 35

CLAIMS:

CLMS(1)

We claim:

1. A method for producing a mutation in a particular region of DNA of a P. haemolytica genome comprising the step of:
isolating said region of the genome from P. haemolytica;
introducing a mutation into said region to form a mutated DNA region;
introducing said mutated, DNA region into a P. haemolytica cell which does not express a PhaI restriction endonuclease, to form transformants; and
screening said transformants for those which have said mutation in said region on chromosomal DNA of said P. haemolytica cell.

CLMS(2)

2. The method of claim 1 wherein said P. haemolytica cell which does not express a PhaI restriction endonuclease is a natural isolate.

CLMS(3)

3. The method of claim 1 wherein said P. haemolytica cell which does not express a PhaI restriction endonuclease is a mutant made by chemical mutagenesis.

CLMS(4)

4. The method of claim 1 wherein said P. haemolytica cell which does not express a PhaI restriction endonuclease is a mutant made by a process comprising:
isolating a region of a genome from P. haemolytica;
introducing a mutation into said region to form a mutated DNA region;
methylating said mutated DNA region with a methylating enzyme which inhibits endonuclease cleavage at a recognition sequence selected from the group consisting of 5'-GATGC-3' and 5'-GCATC-3', to form methylated DNA;
introducing said methylated DNA into a P. haemolvtica cell to form transformants; and
screening said transformants for those which have said mutation in said region on chromosomal DNA of said P. haemolvtica cell.

CLMS(5)